to that effect is provided. Accordingly, entry of the substitute Sequence Listing is also respectfully requested.

If the fee authorized is incorrect or if any other fees are due in connection with this submission, please charge any such fee or credit any overpayment to Deposit Account No. 03-3975.

Respectfully submitted,

PILLSBURY WINTHROP LLP

Date: 11-5-02

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acids in Table 3 (i.e., Gly, Thr, Trp, Ser, Tyr, and Pro) complement each other. For example, each of Arg, Lys, Asn, Asp, Gln, Glu, and His is complementary to Ile; and each of Gly, Thr, Trp, Ser, Tyr, and Pro is complementary to Gly. In other words, each of Arg, Lys, Asn, Asp, Gln, Glu, and His is a complementary amino acid to Ile; and each of Gly, Thr, Trp, Ser, Tyr, and Pro is a complementary amino acid to Gly.

By "hydropathy profile of a peptide or protein" is meant its profile as defined by the hydropathic characters of amino acids in the peptide or protein.

By "complementary peptide" is meant a peptide containing a contiguous amino acid sequence (in the direction from the amino terminus to the carboxy terminus or in the direction from the carboxy terminus to the amino terminus) which complements the amino acid sequence of the target protein/peptide according to the rule set forth above. Any target protein/peptide has a plethora of complementary peptides because each amino acid in the target protein/peptide has a number of complementary amino acids.

By "anti-sense peptide" is meant a peptide encoded by the anti-sense strand of the target gene translated in either 5' to 3' or 3' to 5' directions. For example, if the sense strand of the target gene has 5' CUU GUU CUU UUU 3' encoding a peptide Leu-Val-Leu-Phe, the anti-sense peptide would either be a peptide having the sequence Lys-Lys-Asn-Lys as encoded by the anti-sense strand 5' AAA AAG AAC AAG 3' or a peptide having the sequence Glu-Gln-Glu-Lys as encoded by 5'GAA CAA GAA AAA 3'. The matching pattern in Tables 1-3 shows that the anti-sense strand of a target gene usually encodes complementary peptide to the target protein/peptide. Anti-sense peptides which are complementary to the target protein/peptide are called complementary anti-sense peptides.

By "target complementary library (TCL)" is meant (i) a peptide library containing a collection of complementary peptides of a target protein/peptide, wherein the complementary peptides in aggregation complement all, substantially all (preferably no less than 70%, more preferable no less than 80%, even more preferably no less than 90%), or a significant portion of the target protein/peptide; or (ii) a nucleic acid library containing a collection of nucleic acids encoding the aforesaid complementary peptides. In a preferred embodiment, the complementary peptides are linked to flanking sequences.

By "anti-sense target complementary library (anti-sense TCL)" is meant a target complementary library wherein the complementary peptides are also anti-sense peptides of the target protein/peptide. These anti-sense peptides in combination complement the amino acid sequence of the target protein/peptide in its entirety or

synthetic degenerate BglI deoxyoligonucleotides fragments are synthesized by solid-phase phosphoramidite chemistry (90), and carry the following sequence:

5'-CTGTCAGGGCCCGAGGGGCT(XXX)nGGGGCCGCTGCGGCCTGTCAGG-3' (SEQ ID NO. X)[8] (SEQ. ID. NO. 7)

In the above sequence, n is the number of amino acid residues in the target peptide. The degenerate sequence of (XXX)n is designed by the following principle: hydrophobic amino acids which are complementary to the hydrophilic amino acids in the target peptide are randomly incorporated in the same positions in cTCL; hydrophilic amino acids which are complementary to the hydrophobic amino acids in the target peptide are randomly incorporated in the same positions in sTCL. Slightly hydrophilic amino acids are used in sTCL corresponding to similar residues in the target peptide. The hydrophilic and hydrophobic amino acid residues are defined by their hydropathic scores as given in Tables 1-3.

To synthesize XXX triplets for hydrophilic amino acids, the second base comprises a mixture containing A and G at molar ratio 6:1; the first base consists of equimolar mixture of A, G, and C. Thymine is not included in the mixture because its presence in the first position would give rise to the stop codon TAA or TAG (91). The third base includes only an equimolar mixture of G and C which is designed to favor codons used by *E. coli* to express its most abundant proteins (91).

To synthesize XXX triplets for hydrophobic amino acids, the second base comprises a mixture containing T and C at molar ratio 5:2; the first base consists of mixture containing an A:T:C:G molar ratios of 3:3:3:1. The third base again includes only an equimolar mixture of G and C.

To synthesize XXX triplets for slightly hydrophilic amino acids, the first base consists of an equimolar mixture of A, T, C and G; the second base contains a mixture of G, C, and A molar ration 2:2:1, and third base includes an equimolar mixture of G and C.

The oligonucleotides are purified by denaturing PAGE, their complementary strands are synthesized by Klenow DNA polymerase (91). They are subsequently digested with BglI and ligated with fUSE5 (71, 72, 89).

A cTCL carrying peptides with ten amino acid residues will contain approximately 2.8×10^8 independent clones, which can be completely amplified as a phage display library. A cTCL carrying peptides with eleven amino acids will have approximately 2×10^9 clones.

Synthesize a TCL based on the sequences of FR2 segments and the complementary hydropathy and select peptide ligands by their ability to bind to and disrupt the functions of antibodies with different antigenic specificities. The following examples illustrate how to identify anti-IgE peptides.

A very simplified version of this approach is to identify the FR2 complementary sequences by computer programs (15, 70). Such approach generally produce peptides with binding affinity within the range of 10^3-10^6 M^{-1}. The affinity can be improved by screening a constrained TCL which contains various flanking sequences and cysteins at both sides of the complementary peptides (61).

Example 3: Blocking Human Rhinovirus (HRV) Major Receptor (ICAM-1) with Peptide Ligands

Human rhinoviruses cause about 70% of common cold. ICAM-1 serves as the cellular receptor for majority of HRVs (82). The extracellular part of the ICAM-1 molecule is composed by five immunoglobulin-like domains (D1-D5). Mutational analysis of ICAM-1 has shown that domain D1 contains the primary binding site for rhinoviruses as well as the binding site for its natural ligand lymphocyte function-associated antigen 1 (LFA-1) (83-86). The regions in D1 which have been implicated as the contact sites with HRVs include residues 1, 2, 24-29, 40-49, and 70-77 (83-87). Accordingly, peptide ligands of ICAM-1 targeting these regions may prevent the binding of HRVs to ICAM-1. Suitable peptide targets include, but are not limited to the following:

Residues 1-5: QTSVS (SEQ ID NO. 9)

Residues 24-29: SCDQPK (SEQ ID NO. 10)

Residues 40-49: KELLLPGNNR (SEQ ID NO. 11)

Residues 70-77: PDGQSTAK (SEQ ID NO. 12)

Peptide ligands capable of binding to each of the above target peptides are identified by the following procedure:

(1) Preparing cTCL (or constrained cTCL) for each target peptide, and displaying the library on phage; (2) Immobilizing each target peptide on a solid surface (Alternatively, the D1-D2 part of ICAM-1 molecular is expressed and purified in vitro as described in ref 88, coated onto a solid surface, and used to screen cTCLs); (3) Selecting phage particles by panning as described in detail above; and (4) Recovering the peptide sequences from the selected phage particles.

linking of the IgE molecules. Such peptides target IgE at or near the antigen binding site and prevent antigen binding by causing steric hindrance to the binding site.

It would be preferable that the peptides target the conserved framework regions rather than the hypervariable regions in order to affect IgE molecules of a wide range of antigenic specificities.

TCLT is suitable for finding such peptides. First, it is well-established that the antigen binding site in an antibody molecule (Ab1) can be interfered by anti-idiotype antibodies (Ab2) against this antibody (Ab1). One type of anti-idiotype antibody, Ab2beta, is the direct mirror image of the antibody binding site on Ab1. Another type of anti-idiotype antibody, Ab2gamma, is directed against an idiotype near the binding site and causes steric hindrance to the antigen binding site (62). Second, idiotypes of an antibody are composed by peptide sequences found in both the hypervariable regions (CDRs) and the conserved framework regions of a antibody molecule (62, 69, 70). Therefore, a peptide targeting the conserved region of an idiotype can alter the idiotype and affect the antigen-binding ability of an entire class of antibody, such as IgE, IgG and IgA. Kang et al. have demonstrated the presence of complementary sequences in human immunoglobulin (52).

Peptides affecting antigen-binding ability of human IgE molecules can be identified with TCLT as described in the following examples.

Example 5: Blocking antigen-binding ability of an antibody molecule by targeting the framework 2 (FR2) region of the antibody

Based on their sequences, the heavy chain (H) of an antibody can be classified into six families (V_H1 to V_H6). The general sequences of FR2 region in each family are in the following:

V_H1: W V R/Q Q A P/H/T G/A K/Q G/E/R/A L E/G W M/I G (SEQ ID NO. 13)

V_H2: W I R Q P P G K A L E W L A (SEQ ID NO. 14)

V_H3: W V/I R/H Q A P/Q G K G L/P E/V W/Y/L V S/A/G (SEQ ID NO. 15)

V_H4: W I/V R Q P P G K G L E W I G (SEQ ID NO. 16)

V_H5: W V R Q M P G K G/E L E W M G (SEQ ID NO. 17)

V_H6: W I R Q S P S R G L E W L G (SEQ ID NO. 18)

The sequence of the light chain (L) of an antibody is:

V_L kappa: W Y Q Q K P G Q/K P/S/A P K L L I Y (SEQ ID NO. 19)

Human IgE molecules are mostly composed of heavy chain genes from the V_H5, V_H3, V_H4 and V_H6 families (92,93), therefore the FR2 sequences from these families plus

the FR2 from the light chain serve as the target sequences for an IgE blocking polypeptide in this invention.

The IgE-blocking peptide can be designed directly based on the molecular recognition theory as in the following:

Peptide 1(binds to FR2 in V_H5): P D A L H G P F A Q(or D) L P H P (SEQ ID NO. ~~13~~ 20)

Peptide 2(binds to FR2 in V_H3, V_H4 and V_H6): P D A L G/R G P F A Q/D L P N P (SEQ ID NO. ~~14~~ 21)

Peptide 3(binds to FR2 in V_L kappa chain): P V L L F R P L R G F E E D I (SEQ ID NO. ~~15~~ 22)

To isolate peptides with higher affinity to the FR2 region of IgE, a human IgE anti-sense TCL or comprehensive TCL library can be screen using the above FR2 target sequences.

An alternative way to generate human IgE blocking polypeptide which targets the FR2 region is to screen from a phage display human antibody library. In this case, each peptide encoded by the targeted FR2 sequence is synthesized artificially and used for panning as described below.

Example 6: Construction of a human IgE anti-sense TCL on bacteria phage

The human IgE anti-sense TCL is prepared by using coding sequences of the cDNA clones of human IgE light chain and heavy chain. The cDNAs of human IgE are separated from the vector sequences by agarose gel electrophoresis. Equal amounts of each purified inserts are mixed and used as templates for a random priming reaction.

Approximately 50 ng of the mixed template is boiled with 1 pmol of the primer No. 1 (5'-GACGTGGCCN$_6$-3', N can be A, T, C, or G) (SEQ. ID. NO. 23) for 3 min, cooled on ice, mixed with a reaction mixture containing 10 mM Tris.HCl (pH 7.5), 10 mM MgCl2, 1 mM dithiothreitol, 0.125 mM each dNTP, and 2.5 units of Klenow fragment of DNA polymerase and incubated at 37 °C for 15 min. Then the reaction mixture is boiled and cooled again, another 2.5 units of Klenow fragment is added, and the reaction mixture is incubated at 37 °C for 15 min.

The reaction is stopped by boiling and diluted 1:10 with TE buffer and products are separated from primers using Microcon-100 microconcentrators (Amicon) with two washes of the retained solution with 200 ml of the TE buffer. After two cycles of priming, some reaction products will incorporate primer sequences at both ends. They are

amplified in PCR using primer No. 2 (5'-GGCCGACGTGGCC-3') (SEQ ID NO. 24). The amplified products are precipitated, purified with Microcon-100, cut with sfiI and cloned into the sfiI site of the fUSE5 vector (71, 72). Amplification of the IgE-TCL follows the methods described by Smith and Scott (71), the details which are given in the manual supplied with the fUSE expression kit by Smith (72).

Primer No.1: 5'-GACGTGGCCTGTN6-3' (SEQ ID NO. 25) and primer No. 2: 5'-GGCCGACGTGGCCTGT-3' (SEQ ID NO. 26) are used to generate a constrained IgE-TCL.

Example 7: Selection of the IgE binding complementary anti-sense peptide by panning the human IgE TCL

Human IgE is diluted in PBS to a concentration of 20 mg/ml and is used to coat 3.5 cm wells by incubating for 1 h at 4 °C. The remaining binding sites are saturated by bovine serum albumin (BSA). A portion of the amplified IgE-TCL is first incubated for 2 h at 4 °C in a 3.5 cm well precoated with 1 mg/ml BSA in PBS and 1 mM MnCl2. The phage unbound to BSA are transferred to a similar well precoated with human IgE. After incubation for 1 h at 4 °C, the unbound phage are removed by washing 10 times with PBS buffer containing 0.5% Tween 20. The bound phage are eluted with 0.1 M glycine buffer, pH 2.2, containing 1 mg/ml BSA and 0.1 mg/ml phenol red.

The phages are amplified using the K91kan bacteria and partially purified by precipitation with polyethylene glycol (72). The panning is repeated for two more rounds. Sequences carried by the selected phage are then determined using the Sequenase kit (United States Biochemical) with the primer 5'-CCCTCATAGTTAAGCGTAACG-3' (73) (SEQ ID NO. 27).

Example 8: Characterizing anti-IgE activities of the complementary anti-sense peptides

The peptides selected from the previous step are synthesized and individually tested in the following assays.

Assay 1: Histamine release by passively sensitized basophils.

The procedure is described in detail previously (74). Briefly, reaginic sera are obtained from allergic patients who are allergic to ragweed, rye grass, Chinese elm or other preidentified allergens. Peripheral blood mononuclear cells are isolated from normal individuals by differential centrifugation on Ficoll-Pague (Pharmacia). Aliquots of a cell suspension are incubated with 2- to 10-fold dilution of a reaginic serum, Tris A buffer